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Effects of chronic hyperfiltration on proximal tubule bicarbonate transport and cell electrolytes

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Effects of chronic hyperfiltration on proximal tubule bicarbonate transport and cell electrolytes. The compensatory response to unilateral nephrectomy (UNX) was investigated by a combination of renal clearance, microperfusion, electron microprobe, and morphological techniques. Filtration rate was significantly elevated 21 days following UNX and associated with a marked stimulation of bicarbonate and fluid absorption in the proximal tubule. Analysis of kinetic data of bicarbonate transport demonstrated strong flow-dependent activation of bicarbonate absorption in both control and experimental condition. The bicarbonate level at which half-saturation (apparent K_d) of transport occurred decreased uniformly at higher flow rates, but maximal transport rates (apparent V_{max}) in the proximal tubule doubled in the remnant kidney. The flow dependence of bicarbonate transport in control and experimental conditions can be explained by an apparent unstirred layer effect modifying radial bicarbonate gradients in the tubule. Both Na/H-exchange and electrogenic H secretion contribute to bicarbonate absorption, but only Na/H-exchange increased significantly in proximal tubules of UNX rats. Cell ion concentrations after UNX were unchanged in cortical tubules, consistent with proportionately enhanced apical and basolateral ion transport. Proximal tubule cell rubidium concentration measured after a 30-second rubidium infusion as an index of basolateral Na,K-ATPase activity was unchanged in UNX rats. Inasmuch as cell volume increased significantly (25%), these data are consistent with a proportionate and similar stimulation of rubidium uptake and Na,K-ATPase activity.

The adaptive response of the proximal tubule epithelium to unilateral nephrectomy includes enhanced reabsorption of bicarbonate through increased proton secretion [1–5]. Such Na/H-exchange has also been shown to play a key role in NaCl reabsorption by its coupling to apical Cl/organic anion exchange [6]. A second process, primary active H secretion, also contributes to proximal tubule proton secretion and bicarbonate reabsorption [7–11], but its role in adaptive transport stimulation is poorly understood. Important determinants of proximal bicarbonate absorption include luminal flow rate and the bicarbonate concentration of the tubule fluid [6, 12–16]. In the present investigation we address unresolved problems related to transport stimulation across the proximal tubular epithelium observed in the remaining kidney three weeks after unilateral nephrectomy. We have chosen the *in vivo* microperfusion technique to evaluate tubule transport because, in contrast to studies on isolated tubules and membrane vesicles, it allows a better and more accurate assessment of the interactions between tubule flow rate and individual apical and basolateral transport mechanisms under conditions in which metabolic and hormonal factors can fully display their influence on tubule function.

Specifically, we address three issues. First, previous studies of the relation between tubule flow rate, lumen bicarbonate concentration and proximal bicarbonate absorption established that increased flow rates *per se* activate bicarbonate reabsorption independent of its concentration in the lumen [4, 12, 14, 15]. These findings were interpreted as evidence for an apparent unstirred layer effect which was dissipated at higher lumen flow rates [14]. Accordingly, experiments were carried out to address the following question: Is increased net reabsorption of bicarbonate reabsorption mediated by dissipation of an apparent unstirred layer effect in the brush border membrane? An alternative possibility is transport stimulation by increased number of proton secreting transport molecules.

Second, our study addressed the issue of the contribution of Na/H-exchange and electrogenic hydrogen ion secretion to the stimulation of proximal bicarbonate reabsorption: Is increased net reabsorption of bicarbonate the result of enhanced Na/H-exchange and/or activation of electrogenic H pumps?

Third, we have also investigated the question whether the density of Na,K-ATPase in the basolateral membrane of proximal tubule cells undergoes adaptation in the remaining kidney after nephrectomy. These studies included experiments in which rubidium uptake into single tubule cells as well as morphometric approaches were combined.

Our experiments indicate significant changes of proximal tubule transport after three weeks of unilateral nephrectomy. These involve evidence for increased number of Na/H-exchangers but not of electrogenic H pumps. Flow dependent stimulation of bicarbonate transport was similar to that in control animals. We observed that Na,K-ATPase activity increased proportionately to cell volume so that pump density in hypertrophied proximal tubules remained constant.

Methods

Preparation of animals and experimental protocol

Experiments were performed on male Wistar rats maintained on a standard rat pellet diet (C 1000, Altromin, Lage, Germany; protein content, 173 g/kg) and tap water *ad libitum*. Clearance studies, microperfusion experiments *in situ*, electron microprobe

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analyses, and morphometric studies were carried out in two groups of rats:

Group I. Control rats were sham-operated under ether anesthesia.

Group II. Unilateral nephrectomy (UNX) was carried out under ether anesthesia. Twenty-one days later clearance collections, kidney fixation by retrograde vascular perfusion or microperfusion experiments were performed.

Clearance studies

Twenty-four hour analyses of sodium and potassium excretions were carried out on the day before clearance measurements in awake animals as described previously [17]. The animals were anesthetized by intraperitoneal injection of Inactin (120 mg/kg body wt; Byk-Gulden, Konstanz, Germany) and placed on a thermoregulated operating table (Effenberger, München, Germany) designed to keep the animal's temperature at 37°C. Following a tracheotomy, the right jugular vein and the left femoral artery were cannulated for infusion of saline and monitoring of arterial blood pressure and withdrawal of blood samples. For the determination of left kidney function isotonic saline containing polyfructosan (Laevosan, Linz, Austria; 2.5 g/100 ml) was administered at a rate of 1.0 ml/hr/kg body wt. The left kidney was exposed via a flank incision, placed in a kidney cup and continuously superfused with warm paraffin oil (38°C). Timed urine collections were obtained from a ureteral catheter. Arterial blood samples were collected at the beginning and at the end of the clearance period.

Following the clearance measurements the renal surface was cleaned and rinsed several times with warm (38°C) isotonic saline. Rubidium chloride (0.50 mmol/kg body wt) was infused into the left femoral vein for 30 seconds [18]. The kidney was removed from the animal immediately at the end of the rubidium infusion period and shock-frozen in a 3:1 propane/isopentane mixture (−196°C). Details of the method of rubidium uptake into tubule cells have been published previously in several papers [18, 19].

Preparation of freeze-dried cryosections and electron microprobe analysis

A small piece of the superficial renal cortex was mounted under liquid nitrogen in a specimen holding clamp which was inserted into the cutting arm of a precooled ultracryomicrotome (Ultrotome V, LKB, Bromma, Sweden). The advance of the cryotome was set to deliver 1 μm thick cryosections. The cutting temperature was −90°C. The cryosections were sandwiched between a collodion and a formvar film, freeze-dried overnight at −80°C and 10^{-6} mbar and then rapidly transferred into a scanning transmission electron microscope (DSM 950, Zeiss, Oberkochen, Germany) after warming to about 40°C.

Microprobe analysis of the freeze-dried cryosections was carried out with an energy-dispersive X-ray detector system (Link Systems, High Wycombe, UK) attached to the scanning electron microscope [18]. The acceleration voltage was 20 kV and the probe current 0.3 nA. Small areas (1 to 2 μm^2) were scanned for 100 seconds and the emitted X-rays analyzed in the energy range between 0.2 and 20 keV. The cellular measurements were restricted to the nuclei since previous investigations have shown that X-ray spectra obtained in the cytoplasm may be "contaminated" by contributions originating from extracellular compartments (such as apical vesicles, basolateral infoldings) [20, 21]. Intracellular element concentrations and cell dry weights were obtained as described in detail elsewhere [22].

Table 1. Composition of perfusion solutions

	Solution 1	Solution 2	Solution 3	Solution 4
NaCl	120	90	65	45
$NaHCO_3$	25	55	80	100
KCl	5	5	5	5
$MgSO_4$	1	1	1	1
$CaCl_2$	1.8	1.8	1.8	1.8
Na_2PO_4	1	1	1	1
Glucose	5	5	5	5
Alanine	5	5	5	5
Urea	5	5	5	5

All solutions (mM) contained 0.1% FD & C green dye no. 3 and methoxy-[^{3}H] inulin. Solutions 1 to 3 were gassed with 10% CO_2/90% O_2; solution 4 was equilibrated with 20% CO_2/80% O_2.

Microperfusion experiments

Animals were prepared as described above and single proximal tubules perfused *in situ* by methods previously described [12]. After microperfusion and completion of collection the tubules were filled with high viscosity Microfil (Canton Bio-Medical Products, Boulder, CO, USA). The kidney was excised and stored overnight in deionized water at 4°C. The next day the kidney was macerated for 20 minutes in 25% NaOH allowing dissection of the latex casts and measurement of the length of the perfused tubule. Segments below 1 mm were excluded.

The composition of the different perfusion solutions is shown in Table 1. Proximal tubule bicarbonate absorption was studied at various rates of perfusion (15, 30 and 50 nl/min) or at different bicarbonate concentrations (25, 55, 80, 100 mM) [12, 14, 23]. The bicarbonate-containing perfusion solutions were bubbled immediately before use either with 10% CO_2/90% O_2 (solutions 1, 2 and 3, Table 1) or 20% CO_2/80% O_2 (solution 4, Table 1).

The total [CO_2] in the perfusion solutions and collected fluids was determined by microcalorimetry as described previously [24]. Collected samples were stored under mineral oil preequilibrated with a solution containing 100 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and 48 mM $NaHCO_3$ at 10% CO_2. Aliquots (10 nl) of perfusion fluids and collected perfusates were compared with 5, 10, 15, 25, 50, 60 and 80 mM sodium carbonate standards. The fluid volume of the samples was measured in a constant-bore glass capillary. The radioactivity of ^{3}H-inulin (New England Nuclear, Boston, MA, USA) of the samples was measured in a liquid scintillation counter. Net fluid absorption (J_V) and net flux of HCO_3 (CO_2) were calculated by equations previously published [13, 24]. The following equation was used to estimate the rate of proton secretion (J_H) [13, 24]:

$$J_H = J_{HCO_3}^{net} - J_{HCO_3}^{pass},$$

$$\text{where } J_{HCO_3}^{pass} = P_{HCO_3} \times \Delta HCO_3$$

Bicarbonate permeability (P_{HCO3}) was calculated from the measured transepithelial concentration differences of bicarbonate (driving force) and bicarbonate reabsorption (passive transport). To minimize non-passive bicarbonate absorption acetazolamide

(2 mg/kg body wt) was administered as an i.v. bolus. In addition, it was added to the perfusion solution which had the following composition: NaCl 120 mM, $CaCl_2$ 1.5 mM, raffinose 55 mM, acetazolamide 0.5 mM. Because the transepithelial potential difference (PD) is low in the proximal convoluted tubule, its effect on passive transepithelial bicarbonate flux is negligible. Hence, the transepithelial PD was disregarded when calculating P_{HCO3} [14]. Transepithelial bicarbonate permeabilities were calculated according to the following equation [13, 14]:

$$P_{HCO_3} = -J_{HCO_3} / \Delta HCO_3$$

where ΔHCO_3 is the log mean transepithelial concentration gradient between lumen and peritubular fluid.

Ethyl-isopropylamiloride (EIPA) was provided by Dr. Lang (Hoechst AG, Frankfurt, Germany). We are also grateful for a gift of bafilomycin A1 from Prof. K. Altendorf, Department of Microbiology, University of Osnabrück (Osnabrück, Germany).

Chemical analyses

Sodium and potassium concentrations in serum and urine were measured by flame photometry (IL, Lexington, MA, USA). Polyfructosan in serum and urine was determined by the anthrone method of Führ, Kaczmarczyk and Krüttgen [25]. Polyfructosan clearance and the fractional excretion of potassium and sodium were calculated using standard formulae. Blood pH and CO_2 were determined in a pH/blood gas analyzer (model 168; Corning Inc., Corning, NY, USA).

Stereologic analysis

Kidneys of sham-operated ($N = 4$) and unilaterally nephrectomized ($N = 6$) rats were fixed by retrograde vascular perfusion via the abdominal aorta and prepared for light and electron microscopy as described in detail elsewhere [26]. Semi-thin sections of plastic-embedded specimens of cortical tissue were analyzed for volumes and lengths of proximal and distal convoluted tubule epithelium, as well as of cortical collecting duct epithelium. In the first step, volume (V_v) and length (L_v) densities of cortical nephron segments were determined by point counting [27]. In the second step, the relative volume and length estimates were related to the absolute volume of the renal cortex. The latter was determined by a procedure involving: (a) measurement of total kidney volume by volume displacement following perfusion fixation; (b) sectioning of the organs into five equidistant slices perpendicular to the organ's long axis and embedding in paraplast; (c) selection of one 5-μm section from each of the five tissue slabs for staining with hematoxylin/eosin; and (d) point counting for determining V_v of the renal cortex for calculating its absolute volume.

Presentation of data and statistical analysis

Statistical analysis of the microperfusion data was performed using the unpaired Student's *t*-test. A comparison of the EIPA- and bafilomycin-sensitive transport rates was carried out using an approach described by Eisenberg and Gage [28]. The cell element concentrations and the cell dry weights are presented in mmol/kg wet wt and in g/100 g wet wt. The data are given as means ± SEM. The mean intracellular concentration of sodium, rubidium and chloride obtained in a specific tubule cell type in one kidney was taken as a single data point and used for statistical evaluation. In general, significance of differences between the means was evaluated by Student's *t*-test. Statistical analysis of the stereologic data obtained, however, was performed using the non-parametric, two-tailed rank sum test described by Mann and Whitney [29]. The criterion for statistical significance was $P < 0.05$.

Table 2. Blood values and parameters of kidney function in sham-operated controls and in unilaterally nephrectomized animals (UNX) 21 days after removal of the right kidney

	Controls	UNX
Serum sodium concentration *mmol/liter*	139.7 ± 1.3	141.0 ± 0.7
Serum potassium concentration *mmol/liter*	4.5 ± 0.1	4.4 ± 0.1
Hematocrit %	46.1 ± 0.5	47.6 ± 0.7
Urine flow rate *μl/min/100 g body wt*	1.2 ± 0.2	1.7 ± 0.1[a]
Glomerular filtration rate *ml/min/100 g body wt*	0.48 ± 0.02	0.69 ± 0.03[a]
Sodium excretion *nmol/min/100 g body wt*	27.6 ± 8.0	60.8 ± 18.9
Potassium excretion *nmol/min/100 g body wt*	320.6 ± 29.3	495.1 ± 41.9[a]
Fractional sodium excretion %	0.04 ± 0.01	0.06 ± 0.02
Fractional potassium excretion %	14.8 ± 1.4	16.7 ± 1.5
Body weight *g*	259.3 ± 2.3	258.2 ± 6.1
No. of animals	6	10

Values are means ± SEM.
[a] Significantly different from corresponding control value

Results

Effects of unilateral nephrectomy on glomerular filtration rate, electrolyte excretion and acid-base parameters

Our data on the daily rates of excretion of urine, sodium and potassium by the remaining kidney (not shown) confirm previous results [4, 30–32] that unilateral nephrectomy leads to prompt and effective functional compensation by the remaining kidney.

Table 2 provides further information on urine flow rate, GFR and renal electrolyte excretion following unilateral nephrectomy after 21 days. Control data from sham-operated animals are included for comparison. All data were obtained in anesthetized animals in acute experiments. It is apparent that most values including body wt, mean arterial blood pressure and, in particular, mean serum sodium and potassium concentrations remained unchanged. Values of single kidney GFR, urine flow rate and absolute excretion rates of sodium and potassium were increased, although the value for sodium excretion did not reach statistical significance. In a similar model in which kidney function was evaluated two to four weeks after unilateral nephrectomy, it was also found that serum sodium and potassium concentrations did not differ from control values [33]. It is noteworthy that single kidney GFR was elevated by some 40%, a value comparable to results obtained by other laboratories [3, 4].

Analyses of arterial blood gases and urinary pH and bicarbonate concentrations revealed no significant changes after unilateral nephrectomy. The following values were obtained in control animals: blood pH, 7.39 ± 0.02 ($N = 12$); blood P_{CO2}, 44.3 ± 2.7 ($N = 12$) mm Hg; blood [HCO_3], 25.9 ± 0.7 ($N = 12$) mmol/liter; urine pH, 6.02 ± 0.11 ($N = 12$); urine [HCO_3], 0.3 ± 0.2 ($N = 4$) mmol/liter.

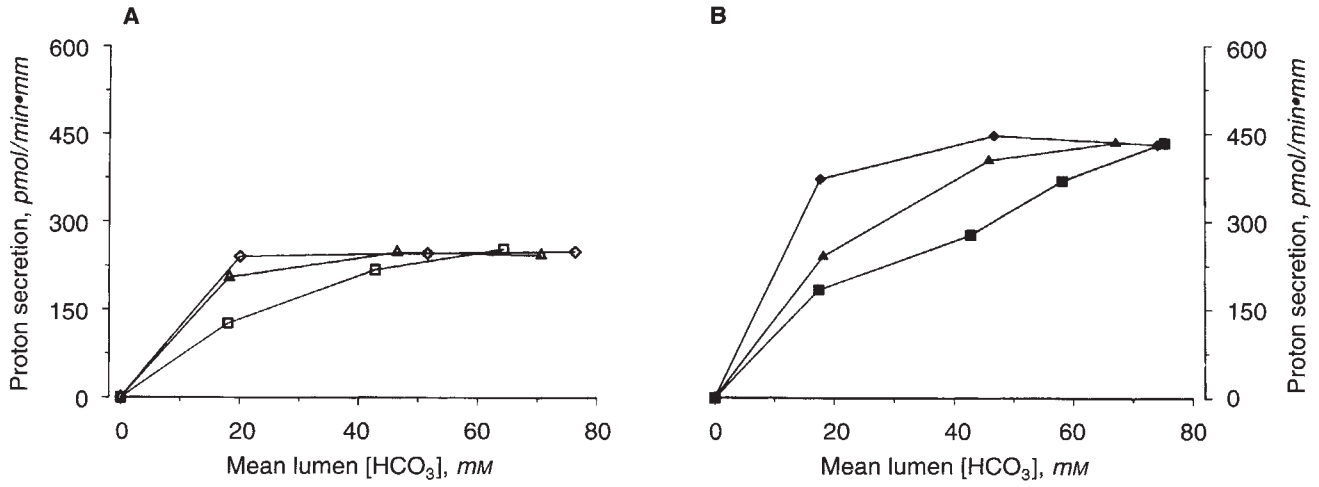

Fig. 1. *Relationship between proton secretion and mean lumen bicarbonate concentration at different perfusion rates in* (**A**) *control and* (**B**) *UNX rats.* Symbols are: (♦, ◇) 50 nl/min; (▲, △) 30 nl/min; (■, □) 15 nl/min.

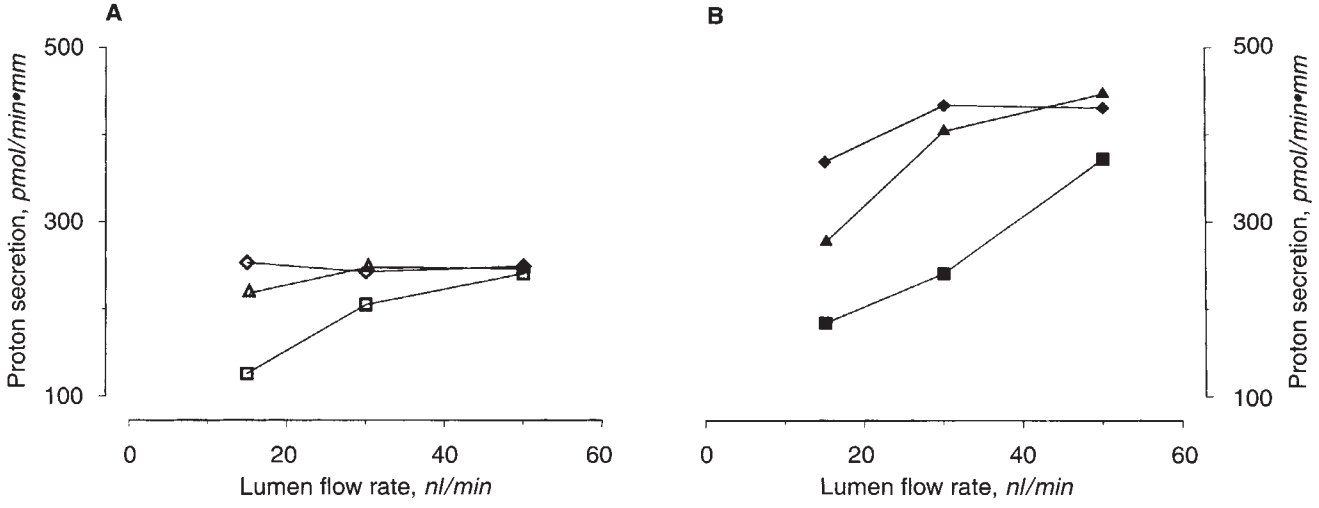

Fig. 2. *Relationship between proton secretion and mean lumen perfusion rate at different bicarbonate concentrations of the perfusion solutions in* (**A**) *control and* (**B**) *UNX rats.* Symbols are: (♦, ◇) 50 n/min; (▲, △) 30 nl/min; (■, □) 15 nl/min.

Effects of unilateral nephrectomy on proximal bicarbonate transport

The results of perfusion studies in which segments of proximal tubule were perfused at flow rates ranging from 15 to 50 nl/min at bicarbonate concentrations between 25 and 100 mM are summarized graphically in Figures 1 and 2. A detailed summary of the measured values and of the calculated data are given in Table 3. These Figures and Table 3 also contain results from unilaterally nephrectomized animals.

Confirming previous results [12, 14, 23] we observed a marked increase of bicarbonate absorption with enhancement of perfusion rate in sham-operated controls. When corrected for passive bicarbonate fluxes and expressed as net H secretion, this effect is particularly striking in the lower range of bicarbonate concentrations from 10 to 40 mM. In contrast, proton secretion rates tend to saturate at higher bicarbonate concentrations (Fig. 1). It should also be noted that saturation of proton secretion is reached at lower mean lumen bicarbonate concentrations as lumen perfusion rates increase. Similar findings were obtained by several investigators [14, 15, 23].

Table 3 and Figure 1 provide also information on bicarbonate absorption and proton secretion in nephrectomized animals. Two points deserve notion. First, proton secretion mediating bicarbonate absorption is significantly increased over the whole range of lumen bicarbonate concentrations (Table 3, Fig. 1) and lumen flow rates (Table 3, Fig. 2). Second, with increasing flow rate half-saturation appears to be reached at lower lumen bicarbonate concentrations. We note from inspection of Figure 1 that in control animals, at a flow rate of 15 nl/min, half-saturation of proton secretion is reached at about 30 mM lumen bicarbonate concentration whereas at a lumen flow rate of 50 nl/min half-saturation occurred at bicarbonate concentrations less than 20 mM. In general, a similar relationship between half-saturation of proton secretion and lumen flow rate was noted in uninephrectomized animals (Fig. 1B).

With regard to the mechanism of stimulation of bicarbonate

Table 3. Effects of flow rate and lumen bicarbonate concentration on net bicarbonate absorption and hydrogen ion secretion in proximal convoluted tubules of control and UNX rats

Groups	N	V_o	$[HCO_3]_p$	$[HCO_3]_c$	$[HCO_3]_m$	$[HCO_3]_{pl}$	Length	J_V	J_{HCO_3}	J_H	Slope
		nl/min	*mM*				*mm*	*nl/min/mm*	*pmol/min/mm*		
Controls	9	15.10 ± 0.04	24.8 ± 0.6	12.0 ± 1.8	18.1 ± 0.9	26.1 ± 0.6	1.96 ± 0.12	1.41 ± 0.28	120.3 ± 14.3	126.2 ± 14.7	−6.88
UNX	9	15.10 ± 0.03	24.9 ± 0.1	10.0 ± 0.8	17.4 ± 0.4	25.7 ± 0.9	1.62 ± 0.07	2.74 ± 0.29[a]	175.6 ± 8.7[a]	184.1 ± 9.0[a]	−9.02
Controls	12	30.14 ± 0.03	25.1 ± 0.5	13.5 ± 1.8	19.3 ± 1.1	25.9 ± 0.5	2.21 ± 0.22	2.19 ± 0.35	199.7 ± 21.2	205.0 ± 21.4	−5.25
UNX	11	30.09 ± 0.04	24.9 ± 0.1	11.5 ± 1.2	18.1 ± 0.7	25.0 ± 0.9	2.34 ± 0.19	3.97 ± 0.31[a]	235.4 ± 17.5	241.2 ± 18.0	−4.06
Controls	9	50.11 ± 0.04	24.8 ± 0.6	15.4 ± 1.0	20.1 ± 0.4	26.1 ± 0.6	2.18 ± 0.09	1.82 ± 0.34	235.9 ± 31.9	240.7 ± 31.6	−4.78
UNX	8	50.10 ± 0.04	24.9 ± 0.1	10.3 ± 1.4[a]	17.6 ± 0.7	24.8 ± 1.0	2.27 ± 0.21	5.08 ± 0.69[a]	366.8 ± 18.6[a]	372.5 ± 18.8[a]	−4.96
Controls	10	15.33 ± 0.07	56.4 ± 0.9	28.2 ± 1.7	42.9 ± 1.1	24.5 ± 0.4	2.20 ± 0.22	0.55 ± 0.12	230.9 ± 22.4	218.3 ± 22.0	−8.85
UNX	6	15.21 ± 0.09	59.6 ± 0.1	26.1 ± 3.6	42.9 ± 2.0	26.1 ± 1.2	2.15 ± 0.22	1.79 ± 0.45[a]	294.7 ± 28.6[a]	277.9 ± 28.2[a]	−10.19
Controls	8	30.42 ± 0.07	57.3 ± 0.7	41.4 ± 1.6	46.5 ± 1.5	25.3 ± 0.2	2.60 ± 0.25	1.11 ± 0.31	261.7 ± 18.0	248.1 ± 18.1	−7.10
UNX	8	30.12 ± 0.07	59.7 ± 1.1	31.8 ± 2.7[a]	45.8 ± 1.0	25.1 ± 1.0	2.41 ± 0.23	2.44 ± 0.35[a]	442.6 ± 46.6[a]	404.7 ± 47.3[a]	−6.14
Controls	8	50.11 ± 0.20	56.4 ± 1.0	45.1 ± 2.8	51.6 ± 1.9	25.2 ± 0.2	2.18 ± 0.14	0.67 ± 0.09	264.1 ± 15.2	246.2 ± 16.2	−5.47
UNX	8	50.12 ± 0.07	59.7 ± 1.1	33.7 ± 3.1[a]	46.7 ± 1.3	25.1 ± 1.0	2.51 ± 0.18	2.10 ± 0.45[a]	455.9 ± 31.7[a]	447.2 ± 29.0[a]	−6.62
Controls	7	15.04 ± 0.03	83.1 ± 1.7	45.7 ± 3.3	64.4 ± 2.3	29.2 ± 0.8	2.42 ± 0.30	0.43 ± 0.14	277.5 ± 34.9	253.5 ± 33.7	−14.34
UNX	7	15.05 ± 0.03	85.9 ± 2.2	32.8 ± 4.2[a]	58.1 ± 1.7	28.9 ± 0.3	2.11 ± 0.34	1.17 ± 0.20[a]	386.2 ± 25.5[a]	369.7 ± 22.5[a]	−13.99
Controls	6	30.07 ± 0.03	85.2 ± 1.8	64.5 ± 5.4	70.7 ± 3.2	28.9 ± 0.9	2.83 ± 0.29	0.47 ± 0.17	265.9 ± 26.3	242.8 ± 29.4	−8.51
UNX	8	30.05 ± 0.03	85.9 ± 2.2	51.9 ± 2.5[a]	67.1 ± 2.4	28.9 ± 0.3	2.64 ± 0.33	0.90 ± 0.21	461.0 ± 49.0[a]	434.3 ± 39.2[a]	−6.98
Controls	7	50.05 ± 0.04	84.4 ± 1.7	69.3 ± 2.9	76.4 ± 2.3	28.7 ± 0.8	2.98 ± 0.27	0.65 ± 0.19	285.9 ± 20.8	249.0 ± 22.6	−4.28
UNX	8	50.05 ± 0.03	85.9 ± 2.2	62.4 ± 2.2	74.1 ± 1.7	28.9 ± 0.3	2.76 ± 0.26	1.37 ± 0.24[a]	504.4 ± 37.4[a]	431.1 ± 38.8[a]	−5.10
UNX	8	14.96 ± 0.02	99.6 ± 0.5	47.2 ± 4.5	75.3 ± 2.1	28.3 ± 0.8	2.07 ± 0.13	2.08 ± 0.29	474.3 ± 34.7	434.5 ± 33.9	−23.71

Values are means ± SEM. Abbreviations are: V_o, perfusion rate; $[HCO_3]_p$, bicarbonate concentration of perfusion solution; $[HCO_3]_c$, bicarbonate concentration of collected fluid; $[HCO_3]_m$, mean lumen bicarbonate concentration; $[HCO_3]_{pl}$, plasma bicarbonate concentration; slope, slope of the linear regression relating total CO_2 concentration in collected perfusate with tubule length, the more negative the value the steeper the decline of lumen bicarbonate concentration along the proximal convoluted tubule.

[a] Significantly different from corresponding control value ($P < 0.05$)

absorption, concentration changes of bicarbonate have to be considered. In particular, it is likely that the concentration of bicarbonate decreases more rapidly along the proximal tubule at lower flow rates. This can be explained by the enhancement of J_{HCO3} due to longer exposure of the fluid to proximal tubule cells as the flow rate declines [5, 14, 15]. Accordingly, bicarbonate transport might be accelerated at the higher flow rates by maintaining higher lumen bicarbonate concentrations. Relevant data obtained in both control and nephrectomized animals are summarized in Table 3 (column "slope"). Confirming previous results [12, 14], we found in the control group that the lumen bicarbonate concentration declined more rapidly, reflected by higher values of the negative slopes, when tubules were perfused at a lower perfusion rate. For instance, the slope in control animals (perfusion rate 15.1 nl/min, bicarbonate concentration 24.8 mM) was −6.88 whereas the value at a flow rate of 50.1 nl/min was −4.78. A similar comparison indicates that the slope relating bicarbonate concentration to tubular length also declined in uninephrectomized animals when flow rate was increased. Thus, at a concentration of bicarbonate in the lumen of 24.9 mM the slope declined from −9.02 at a perfusion rate of 15.1 to −4.96 at a flow rate of 50.1 nl/min. It should be noted though, that despite stimulation of proximal bicarbonate absorption the decline of bicarbonate concentration was similar in kidneys of unilaterally nephrectomized rats compared with control kidneys. This functional pattern is expected because both bicarbonate and fluid absorption are significantly increased in the experimental group.

The P_{HCO3} was measured in two experimental conditions. In sham-operated animals, P_{HCO3} was $1.76 \pm 0.31 \times 10^{-5}$ cm/sec. This value is similar to that found in previous investigations [13, 34]. A significantly higher value, $3.04 \pm 0.39 \times 10^{-5}$ cm/sec, was observed in UNX rats.

Two transport mechanisms, Na/H-exchange and primary active proton secretion by H-ATPase, have been identified in the apical membrane to contribute to net bicarbonate absorption along the proximal tubule [6]. To examine to what extent these transport mechanisms participate in the stimulation of bicarbonate transport in uninephrectomized animals, inhibitor studies with EIPA and bafilomycin were carried out [35, 36]. The results of these studies are summarized in Figure 3. Whereas under control conditions EIPA reduced J_H from a control value of 218.3 ± 2.2 to 152.2 ± 26.8 pmol/min/mm (N = 10 and 8, respectively), comparative values following unilateral nephrectomy were a decline from a control value of 277.9 ± 28.2 to 120.8 ± 34.2 pmol/min/mm (N = 8 and 5, respectively). Bafilomycin diminished J_H to 112.6 ± 4.5 and 132.8 ± 38.3 pmol/min/mm in control conditions and after unilateral nephrectomy (N = 5 and 4, respectively). It is apparent that inhibition of both Na/H-exchange and/or H-ATPase activity reduces net bicarbonate absorption. A statistical analysis of the relative contribution of EIPA-dependent J_{HCO3}, that is, $J_{Na/H\text{-}exchange}$, and of bafilomycin-dependent J_{HCO3}, that is, $J_{H\text{-}ATPase}$, to total bicarbonate absorption in control and UNX animals shows that only the Na/H-exchange component is significantly increased ($P < 0.05$). Although the bafilomycin-sensitive component of J_{HCO3} also increased, this change did not reach statistical significance.

Relation between bicarbonate and fluid absorption

Our experiments confirm that bicarbonate absorption plays a significant role in fluid transport across the proximal tubule

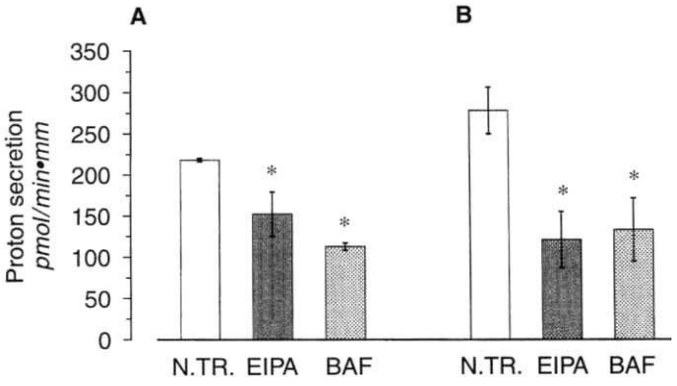

Fig. 3. *Effect of EIPA (10^{-5} M) and bafilomycin (BAF, 10^{-7} M) on hydrogen ion secretion in* (**A**) *control and* (**B**) *UNX rats.* The tubules were perfused at 15 nl/min with a perfusion solution containing 55 mM bicarbonate. Abbreviation N.TR. is not treated. Data are mean ± SEM. *Significantly different from corresponding control (N.TR.) value.

epithelium [37–41]. The data given in Table 3 provide insight into the relationship between net bicarbonate and net fluid absorption. Several points deserve notion. First, it is apparent that at each set of experiments with a given bicarbonate concentration in the perfusion fluid, both J_H and J_V increase uniformly in tubules of UNX rats. For instance, at a perfusion rate of about 15 nl/min and an initial bicarbonate concentration of about 25 mM J_H increases from 126.2 to 184.1 pmol/min/mm as J_V is augmented from 1.41. to 2.74 nl/min/mm. Coordinated increments of hydrogen ion-mediated bicarbonate absorption and fluid transport were also noted at flow rates of 30 and 50 nl/min. A similar tight relationship between increments in bicarbonate and fluid transport was also apparent at the bicarbonate concentrations of about 58 and 84 mM (Table 3).

A second point concerns the relationship between absolute lumen bicarbonate concentration and J_V. In general, fluid absorption at a given flow rate declined with increasing lumen bicarbonate concentration. This can be explained by the decreasing transepithelial concentration difference of bicarbonate which lowers the driving force for solvent drag-dependent fluid movement [34, 38, 41]. A representative example in the control group is given by the comparison of J_H and J_V at a flow rate of 15 nl/min. As the concentration of bicarbonate in the lumen was increased in these experiments from about 25 to 56 and 83 mM, J_V declined from 1.41 to 0.55 and 0.43 nl/min · mm. A similar relationship between increasing lumen bicarbonate concentration and decline in J_V was also observed at higher flow rates and in kidneys of UNX animals.

Effects of unilateral nephrectomy on cell ion composition and rubidium uptake

Sodium, rubidium and chloride concentrations of tubule cells of the superficial renal cortex are summarized in Figure 4. Control values are compared with results obtained 21 days after unilateral nephrectomy. Neither sodium, rubidium nor chloride concentrations were significantly altered in any of the tubule cells analyzed (Fig. 4). These results differ from several experimental conditions in which significant alterations in proximal tubule cell composition had been noted [17, 42, 43].

The present finding that element concentrations of proximal tubule cells are not altered after unilateral nephrectomy differs

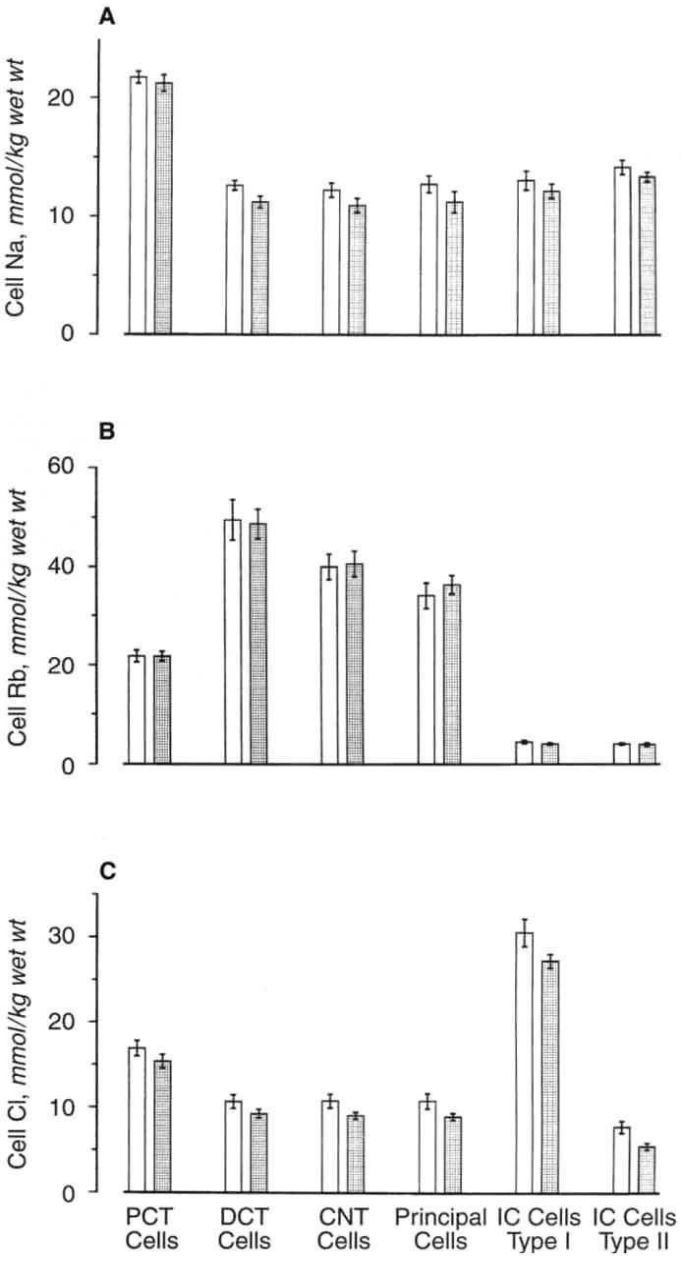

Fig. 4. *Intracellular sodium, rubidium and chloride concentrations (in mmol/kg wet wt) of proximal convoluted tubule (PCT) cells, distal convoluted tuble (DCT) cells, connecting tubule (CNT) cells, principal and type I and type II intercalated (IC) cells of control (open bars) and UNX (shaded bars) rats.* Data were obtained in animals which had received a 30 seconds i.v. rubidium infusion; mean ± SEM.

from observations by Pollock and coworkers [44]. These authors noted decreased sodium and rubidium and increased potassium concentrations in proximal tubule cells of UNX animals. A straightforward explanation for this discrepancy is not apparent. It should be noted, however, that the studies of Pollock and associates were carried out four to six weeks after UNX when left kidney GFR was even higher than in our model.

Confirming previous results we have observed in the present study two types of intercalated cells with different cell chloride

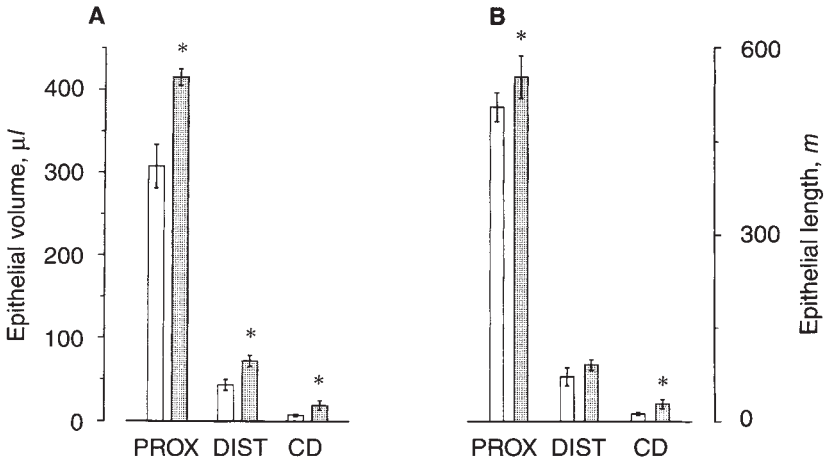

Fig. 5. *Absolute volumes (A) and lengths (B) of cortical tubule segments of control (open bars) and UNX (shaded bars) rats.* Abbreviations are: PROX, proximal tubule epithelium; DIST, distal tubule epithelium; CD, collecting duct (including the connecting tubule). Data are mean ± SEM. *Significantly different from corresponding control value.

concentrations [45]. Chloride-rich intercalated cells, defined in the present study as type I had a cell chloride concentration of 30.6 ± 1.6 in control rats and of 27.3 ± 0.8 mmol/kg wet wt in UNX animals. In low-chloride intercalated cells, defined in the present study as type II, the respective values were 7.8 ± 0.7 and 5.5 ± 0.4 mmol/kg wet wt. In a previous study we have suggested the possibility that cells with high chloride concentrations represent proton-secreting alpha-intercalated cells, whereas those with low cell chloride concentration are more likely to be beta-intercalated cells [45].

Quantitative morphology

The kidney volumes obtained after perfusion fixation were 1188 ± 67 μl in sham-operated controls and 1763 ± 50 μl in UNX animals, the cortical volumes 647 ± 39 and 960 ± 33 μl, respectively. Estimates of volume and length densities of proximal tubular, distal tubular and collecting duct epithelium were referred to the above cortical volume. Relevant data are given in Figure 5. The total volume of the proximal tubular epithelium was increased by 35% 21 days after UNX. Those of the distal tubular epithelium and collecting duct epithelium were increased by 67% and 159%, respectively. Changes in tubular length of these nephron segments (per kidney cortex) were far less prominent for the proximal and distal tubule segments. A marked increase (by 120%) was noted for the cortical collecting duct.

Discussion

The present study confirms and extends previous observations that unilateral nephrectomy stimulates proximal tubule $NaHCO_3$ and NaCl absorption [1, 4, 33, 44]. Both transport studies as well as experiments using brush border vesicles have shown that Na/H-exchange is sharply increased following chronic hyperfiltration that occurs after loss of renal mass [2–4, 46]. The secretion of hydrogen ion is the main process mediating bicarbonate transport. Our experiments address several unresolved issues concerning the mechanisms underlying stimulation of proximal tubule bicarbonate transport. Net bicarbonate absorption along the proximal tubule is critically dependent on transcellular, active proton secretion and on passive paracellular HCO_3 backleak from the peritubular capillaries. Proton secretion is accomplished by two mechanisms localized in the apical membrane [6]. These include, first, secondary active Na/H-exchange that couples entry of one proton to exit of one sodium ion and, second, primary active, ATP-dependent H translocation.

When allowance is made for the passive contribution of bicarbonate efflux to net absorption from the lumen, H secretion increases with flow rate and saturates between lumen bicarbonate concentrations of 45 to 60 mM [12, 14, 15, 23]. Microperfusion studies have also demonstrated that the decline of lumen bicarbonate concentration along the proximal tubule is smaller as flow rate increases [12, 14]. Accordingly, it is possible that part of the stimulation of bicarbonate absorption with higher flow rates is caused by the increase in mean lumen bicarbonate concentration.

However, studies in perfused proximal tubules [4, 12, 14] including experiments in which proxmal tubule cell pH was monitored [15], and in cultured proximal tubule cells [47] show that H secretion increases independent of changes in lumen bicarbonate concentration following acceleration of apical perfusion rate.

Although Na/H-exchange has been demonstrated to be the main mechanism responsible for bicarbonate absorption across the proximal tubule epithelium, electrogenic ATP-dependent H secretion has also been shown to participate in proximal acidification [6–11, 48]. The present experiments confirm that in both control and experimental groups significant fractions of proximal bicarbonate transport can be inhibited by bafilomycin, a specific inhibitor of vacuolar H-ATPase. The present findings indicate that stimulation of bicarbonate reabsorption in UNX-animals is mediated by preferential activation of Na/H-exchange, whereas the component of ATP-dependent H translocation remains unchanged. A combination of electrophysiological techniques with stationary microperfusion studies in which lumen pH changes were monitored in chronically adapted proximal tubules provided also evidence of an increased number of hydrogen ion transporters [5]. The presence of apical H-ATPase activity was also ascertained [5].

An important observation in our studies was the marked flow dependence of bicarbonate absorption that was noted in the remaining kidney after unilateral nephrectomy. This behavior was also seen in control kidneys and has been reported previously by several investigators [12, 14, 15, 23]. We believe that two factors, flow-dependent changes in lumen bicarbonate concentrations and transepithelial bicarbonate permeability, cannot fully account for the significant transport stimulation. First, flow-dependence of

bicarbonate absorption in control kidneys is limited to mean bicarbonate concentrations below 40 mM. Inspection of Table 3 shows that in this concentration range an increase in flow rate produces only a minor elevation of the mean lumen bicarbonate concentration. For instance, at a bicarbonate concentration of the perfusion solution of 25 mM, raising perfusion rate from 15 to 50 nl/min elevated mean lumen bicarbonate concentration only by 2 mM. At unchanged perfusion rate (15 nl/min) an increase in bicarbonate concentration of such magnitude could at best account for 10% of the observed increment of bicarbonate transport that uniformly takes place when flow rate is elevated from 15 to 50 nl/min.

The situation is qualitatively similar in proximal tubules of remnant kidneys. Transport of bicarbonate and hydrogen ions was dramatically stimulated with increasing flow rate achieving much higher values of maximal transport rates. Particularly at the higher flow rates, bicarbonate absorption reached values about twice the magnitude observed in control kidneys. Considering the possible transport stimulation that could have resulted from flow-dependent augmentation of mean lumen bicarbonate concentration, it is safe to conclude that this mechanism plays only a minor role in the observed enhancement of bicarbonate absorption.

A second factor that could be involved in the observed stimulation of bicarbonate transport concerns flow-dependent changes in bicarbonate permeability. Two components of proximal bicarbonate transport have been identified, one dependent on hydrogen ion secretion and a second one dependent on passive bicarbonate diffusion [13, 14, 23, 34]. At low lumen bicarbonate concentrations, below plasma levels, passive bicarbonate influx tends to reduce bicarbonate efflux mediated by H secretion, whereas at bicarbonate levels above plasma net bicarbonate transport exceeds that accounted for by H secretion [6, 13]. Several studies have shown that the bicarbonate permeability of the proximal tubule is relatively low [13, 34]. Accordingly, passive bicarbonate fluxes can be shown to play only an minor role in modifying net bicarbonate transport the latter being mediated mainly by active hydrogen ion secretion. In agreement with others [14, 34] we calculated that passive bicarbonate movement can account only for 2 to 14% of net bicarbonate transport (Table 3). Obviously, the effect of the passive component of bicarbonate transport becomes largest at the highest lumen bicarbonate concentrations when both active hydrogen ion secretion and bicarbonate diffusion contribute to net bicarbonate absorption.[1]

From these considerations it may safely be concluded that the major effect of increasing flow rate is on active hydrogen ion secretion. We have confirmed in the present studies the observation by Alpern et al [4, 14], Preisig [15] and Liu and Cogan [23] that saturation of proton secretion at high flow rates occurs at lower mean bicarbonate concentrations. Figure 1 shows that this kinetic pattern of flow dependence of bicarbonate transport occurred not only in the control but also in the experimental group. Thus we conclude that stimulation of bicarbonate transport following UNX takes place without modification of the flow-dependent pattern of bicarbonate transport. The significantly higher levels of proton secretion in remnant kidneys occur at all flow rates and are consistent with an increased number of hydrogen ion transporters. Formally, the striking flow dependence of bicarbonate absorption can be explained by a diffusion barrier that becomes less effective with increasing flow [14]. Alpern et al have pointed out that such a variable and flow-dependent diffusion barrier could generate a radial concentration gradient of bicarbonate which dissipates with increasing axial flow rates [14]. At present it is not clear how flow rate could modify an effective diffusion barrier [49–51]. Although an increase in lumen flow rate has been shown to widen the distance between microvilli of proximal tubules [52], it remains unresolved how such geometric changes could optimize access of bicarbonate ions to the H extruding mechanisms in the apical membrane.

No changes in cell sodium or chloride concentrations were observed in proximal convoluted tubule cells or in the various cell types of the distal convolution and initial collecting duct 21 days after UNX. Since sodium absorption in the remaining kidney has been shown to increase both in the proximal tubule and the cortical collecting duct [33, 44, 53], stability of intracellular sodium concentrations in cortical tubule cells of UNX rats indicates that sodium entry across the apical cell membrane and sodium exit across the basolateral cell membrane are precisely adjusted without major changes in cell sodium concentration. While apical sodium entry may proceed passively along a favorable electrical-chemical gradient, sodium transport from cell to interstitium is accomplished predominantly by primary active Na/K(Rb)-exchange. One would thus suspect that Na,K-ATPase activity is increased in the proximal convoluted tubule and cortical collecting duct. Indeed, several studies have demontrated an increase in Na,K-ATPase activity in the proximal and distal convoluted tubule and cortical collecting duct of UNX animals [46, 53–57]. In the present study, however, intracellular rubidium concentrations measured precisely after a 30-second rubidium infusion were similar in tubule cells of control and UNX rats. Given the fact that the volume of tubule cells in the proximal and distal convolution and in the cortical collecting duct were increased in UNX animals by 25, 33 and 17%,[2] unchanged cell rubidium uptake implies that the amount of rubidium entering these cells must have increased in proportion to cell volume. It is most reasonable to interpret these observations by assuming that the density of Na/K-pumps remained constant in both proximal and distal tubule segments.

Conclusions

Chronic hyperfiltration following unilateral nephrectomy induces a sharp increase in proximal sodium, tubule fluid and bicarbonate absorption. Increased bicarbonate absorption after UNX is strongly flow-dependent and V_{max} doubles. Transport stimulation can be explained by augmentation of apical Na/H-exchangers whereas the kinetics of flow-dependent bicarbonate reabsorption remain unaltered. Cell ion concentrations after UNX remain unchanged and indicate proportionally accelerated apical and basolateral ion transport. Data of cell rubidium uptake and tubule cell volume changes are consistent with augmented

[1] Although P_{HCO_3} was not measured at all lumen bicarbonate concentrations, we consider it highly unlikely that P_{HCO_3} increased sufficiently at high bicarbonate concentrations to invalidate our conclusion that P_{HCO_3} changes play only a minor role in explaining the observed flow-dependent changes of J_{HCO_3}.

[2] These values are obtained when the total absolute tubule volumes (in microliter) are referred to tubule lengths (meter), and data of UNX animals are compared with those of controls.

Na,K-ATPase turnover rate that is proportional to the increase in proximal tubule cell volume.

Acknowledgments

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